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Mr. Stephen Johnson, Administrator
U.S. Environmental Protection Agency
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1200 Pennsylvania Ave., N.W.
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Subject: Public Comments on the HPV Challenge Program Test Plan for Cobalt Naphthenate (CASRN 61789-51-3) by Members of the Metal Carboxylates Coalition (OM Group, Inc., The Shepherd Chemical Company and Troy Corporation).

The following comments on the HPV Challenge Program test plan for cobalt naphthenate by members of the Metal Carboxylates Coalition (OM Group, Inc., The Shepherd Chemical Company and Troy Corporation) are submitted on behalf of People for the Ethical Treatment of Animals, the Physicians Committee for Responsible Medicine, the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These health, animal protection, and environmental organizations have a combined membership of more than ten million Americans.

In our August 15, 2006 submission, we requested that EPA reopen the comment period for the metal carboxylates test plans, since, as a result of breaking up the category, the numbers of animals to be used has greatly increased and there are a number of serious scientific and animal welfare concerns that need to be addressed. This is the second set of comments that we have submitted on the new individual test plans.

The sponsoring companies are proposing to conduct: a combined repeated dose test with repro/developmental screen, OECD 422, an *in vivo* micronucleus test and an acute fish toxicity test. If conducted, these tests will cause the suffering and death of approximately 875 animals.

This test plan violates the following terms of the October 1999 agreement among the EPA, industry, and health, animal protection, and environmental organizations, as well as the December 2000 *Federal Register* notice reconfirming that agreement:

2. Participants shall maximize the use of existing and scientifically adequate data to minimize further testing.

5. Participants are encouraged to use in vitro genetic toxicity testing to generate any needed genetic toxicity screening data, unless known chemical properties preclude its use.

Cobalt carboxylates are used as oxidative polymerization catalysts in many industries. Other uses include oxygen scavenger plastics and as adhesion promoters in tire manufacturing. Cobalt naphthenate is used in unsaturated polyester resins, paint drier applications and as a rubber adhesion promoter.

The sponsoring companies note that metal carboxylates readily dissociate into free metal and free acid. The proportion of dissociated salt is dependent on the pH, and the dissociation constant (pKa) is the pH at which 50% dissociation occurs. The pKa values for cobalt naphthenate are reported to be 6.74 and 8.00 as determined in studies conducted by the Metal Carboxylates Coalition. These values indicate that complete dissociation will occur at the physiologically relevant pH of the mammalian stomach (pH 1.2). The sponsoring companies conclude therefore, that when administered orally, the toxicity of cobalt naphthenate is due to the independent action of naphthenic acid and the free cobalt ion. As a result, mammalian toxicity data for naphthenic acid and the free cobalt ion, or its simple metal salts, can serve as surrogate data for cobalt naphthenate. In support of this conclusion, the work of Stopford, et al. (2003)[1] is cited to show that cobalt chloride is similar to, or more bioavailable than, the corresponding cobalt carboxylate salts, thus making the chloride a conservative surrogate in estimating bioavailability and toxicity of the dissociated metal ion.

Existing data is summarized for repeated dose, reproductive and developmental toxicity endpoints for naphthenic acids and cobalt chloride. Multiple studies measuring each endpoint are summarized for cobalt chloride. For naphthenic acids, reliable data is summarized from a 90-day repeated dose study on rats in which ovaries were examined and from a one-generation reproduction study on male rabbits treated dermally. Reliability was not assigned for reproductive and developmental toxicity endpoints from summarized studies on rats. A combined repeated dose test with repro/developmental screen, OECD 422, is proposed for cobalt naphthenate. The theoretical discussion of metal carboxylates dissociation presented in the test plan and summarized above clearly shows that data for naphthenic acids and cobalt chloride can serve as surrogate data for cobalt naphthenate. Although the reproductive and developmental toxicity endpoints for naphthenic acids in rats were published only as a meeting abstract[2], these studies are recent and conducted by the same laboratory (Rogers, et al., 2002) which conducted the summarized repeated dose study.[3] It is conceivable that additional detail from these studies could be obtained which would lead to increased confidence in the reliability of these data, and we urge the sponsoring companies to investigate this possibility. A combined repeated dose test with repro/developmental screen, OECD 422, has already been proposed for naphthenic acids under the Reclaimed Substances: Naphthenic Acids test plan of the American Petroleum Institute (API). We would like to reiterate our comment on the API test plan that additional detail from these same studies would also make this test unnecessary. However, if API proceeds with their proposed test, the sponsors of the current test plan should wait to summarize its results thereby satisfying the reproductive and developmental toxicity endpoints for cobalt naphthenate without additional testing.

An *in vivo* micronucleus test is proposed for cobalt naphthenate. Existing chromosomal aberration data are summarized for sodium naphthenate and cobalt chloride. Once again, data for these dissociation products can serve as surrogate data for cobalt naphthenate thereby satisfying the requirements for this endpoint. The proposed *in vivo* micronucleus test for cobalt naphthenate

is outside the scope of the HPV Challenge Program. An *in vivo* test clearly contradicts the principles laid out for the HPV Program in both the EPA's October 1999 letter to chemical sponsors and its December 2000 Federal Register notice on the program, which state that *in vivo* genotoxicity testing should be conducted only when known chemical properties preclude the use of *in vitro* testing. If an additional chromosomal aberration test is perceived to be necessary, an *in vitro* test, OECD 473, should be conducted on cobalt naphthenate – per the Federal Register instructions – rather than an *in vivo* test which will cause the suffering and death of 80 animals.

A fish acute toxicity test is proposed for cobalt naphthenate. No reliable ecotoxicity data for aquatic plants or invertebrates exist for cobalt naphthenate. The fish test is intended to show whether exposure to cobalt naphthenate will result in large-scale fish death thereby predicting economic loss and ecologic damage. If this exposure kills the food on which fish subsist, it could deplete fish populations even without direct fish toxicity. Since the toxicity of cobalt naphthenate to aquatic plants and invertebrates is still unknown, tests on fish are premature. In addition, ECOSAR and non-animal ecotoxicity tests, such as the DarT test[4] and TETRATOX test[5] should be considered. If a fish acute toxicity test is still perceived to be required, ECVAM's Ecotoxicology Task Force recently published an evaluation of a fish acute threshold (step-down) test concept with the potential to reduce the number of fish used in ecotoxicity testing by 53.6%-71.2%.[6]

In summary, we urge the sponsoring companies to investigate the possibility of obtaining additional details for the Rogers, et al., 2002 studies of the reproductive and developmental toxicities of naphthenic acids. If data from these studies are judged to be reliable, there is no justification for the proposed combined repeated dose test with repro/developmental screen, OECD 422. Further, if the American Petroleum Institute proceeds with their proposed OECD 422 test for naphthenic acids, the sponsors of the current test plan should wait to summarize its results in order to satisfy the reproductive and developmental toxicity endpoints for cobalt naphthenate without additional testing. In addition, the proposed *in vivo* genotoxicity test clearly contradicts the EPA's instructions that *in vivo* genotoxicity testing should be conducted only when known chemical properties preclude the use of *in vitro* testing. We urge the sponsoring companies and the EPA to reject these proposed tests as well as to consider the applicability of the suggested alternatives to the fish acute toxicity test.

Sincerely,

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Research & Investigations

[1] Stopford W., Turner J, Cappellini D, and Brock T. 2003. Bioaccessibility testing of cobalt compounds. J. Environ. Monit. 5(4): 675-680.

[2] Rogers, V.V., M. Wickstrom, K.Liber, and M.D. MacKinnon. 2002.Acute and subchronic mammalian toxicity of naphthenic acids from oil sands tailings. Tox. Sci. 66: 347-355.

[3] Rogers, V.V., M. Wickstrom, K.Liber, and M.D. MacKinnon. 2002. Mammalian toxicity of naphthenic acids derived from the Athabasca Oil Sands (AOS). Toxicologist 66(1-S): 64-5. [meeting abstract]

[4] Nagel, R. 2002. DarT: the embryo test with the zebrafish *Danio rerio*: A general model in ecotoxicology and toxicology. *ALTEX* 19 (Suppl. 1), 38-48.

[5] Schultz, T.W. 1997. TETRATOX *Tetrahymena pyriformis* population growth impairment endpoint: A surrogate for fish lethality. *Toxicological Methods* 7, 289-309.

[6] Jerama, S., et al. 2005. A strategy to reduce the use of fish in acute ecotoxicity testing of new chemical substances notified in the European Union. *Regulatory Toxicology and Pharmacology* 42, 218–224.